Cryopreservation of Adult Male Spring and Summer Chinook Salmon Gametes in the Snake River Basin

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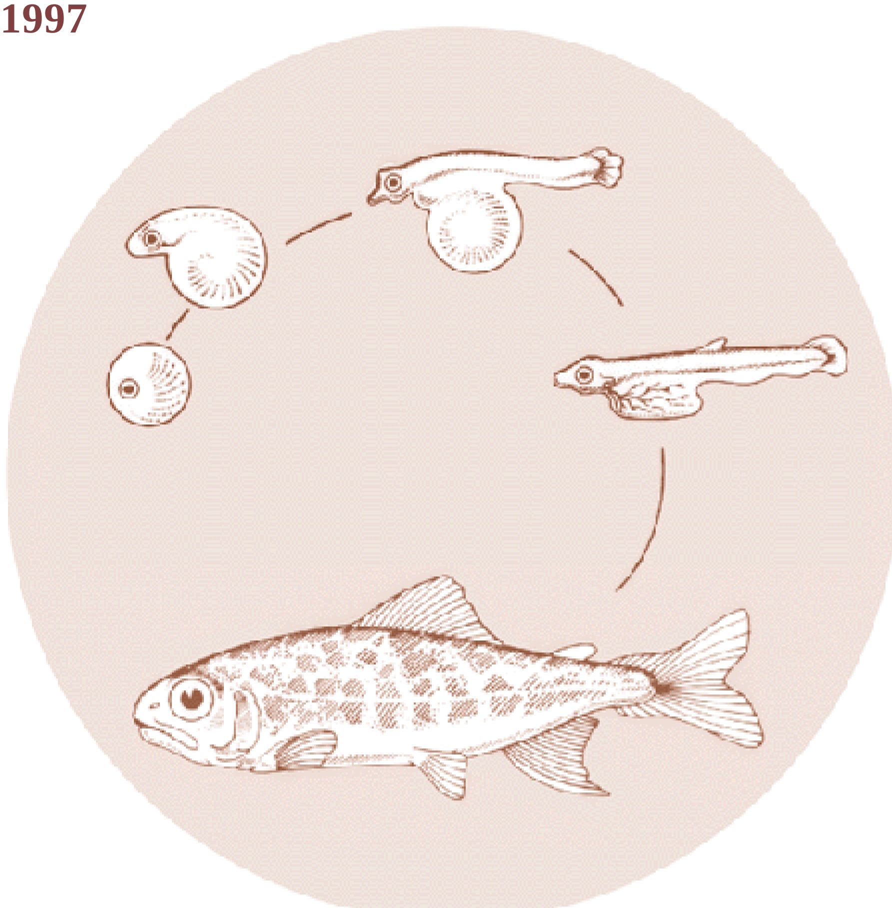
1997



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Cryopreservation of Adult Male Spring and Summer Chinook Salmon Gametes in the Snake River Basin

1997 Annual Report

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ABSTRACT

Chinook salmon populations in the Northwest are decreasing in number. Detrimental conditions causing these decreases can be improved in some cases, but time is required. The best way to ensure availability of a representative genetic sample of the original male population is to establish a germ plasm repository. Cryopreservation of semen is the simplest and most economical means. Cryopreservation provides for a genetic repository and is not a cure for decreasing fish stock problems. The Nez Perce Tribe was funded in 1997 by the Bonneville Power Administration to coordinate and initiate gene banking of adult male gametes from Endangered Species Act (ESA) listed spring and summer chinook salmon in the Snake River basin. In 1997, a total of 189 viable chinook salmon semen cryopreservation samples were taken from the Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River stocks) and Lookingglass Hatchery (Imnaha River stock). A total of 269 cryopreserved samples from Snake River basin spring and summer chinook salmon, from as early as 1992, are in storage at two independent locations, at the University of Idaho and Washington State University.

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INTRODUCTION

Snake River spring and summer chinook salmon spawning aggregates have experienced significant decline in numbers over the past five decades and are now listed as a threatened species under the Endangered Species Act. These declines are due to many different factors. Most are the result of human activities.

Genetic conservation through population protection and monitoring has not been successful. With the constant threat of losing genetic diversity in specific native fish stocks, the establishment of a program for the long-term storage of fish germ plasm would serve as insurance against population collapse and extirpation. One way to ensure that a representative genetic sample of the original population exists, is to establish a germ plasm repository. At present, cryopreservation of semen is the best means of storing fish germ plasma for extended periods of time. Cryopreserved salmonid semen will remain viable for an extended time and can be easily shipped. Ashwood-Smith (1980), Whittingham (1980), and Stoss (1983) has estimated the storage time for fish semen held in liquid nitrogen to be between 200 and 32,000 years. This storage period is more than adequate for a germ plasm repository. The technology for preservation of female gametes is currently not available to fisheries science. Maternal DNA cannot be preserved. Successful research and development in this area would allow the ability to preserve germ plasm components from male and female chinook salmon to preserve future management options.

There are two important factors to be considered when developing a germ plasm repository. First, this is a genetic repository and will not solve population problems of a fish stock that is decreasing. Second, fertility of the stored semen currently is not as great as the fresh semen. The quality of the stored semen is usually a direct reflection of the quality of the sperm that was cryopreserved, and 50 to 80% motility of sperm is considered good. It is desired that fertilization rates using cryopreserved semen in conventional hatchery programs would average 80% or higher. Several limited trials have documented average fertilization rates of 65% using cryopreserved semen (Glen Mendel personal communication). The fertilizing ability of the frozen milt has been tested against controls using fresh milt, and based on the number of eyed eggs, was approximately 60% of that achieved when using fresh milt (Gausen 1991). There is a risk of lower fertilization rates and potential loss of eggs using cryopreserved semen. Lesser fertilization rates may be acceptable where genetic concerns warrant them, such as in captive brood stock programs.

The Nez Perce Tribe initiated chinook salmon cryopreservation activities in 1992. The Lower Snake River Compensation Plan hatchery evaluations program, funded through the U. S. Fish and Wildlife Service has provided a valuable though limited amount of funding for this effort from 1992 through 1996. The Nez Perce Tribe was funded by Bonneville Power Administration in 1997 to coordinate and initiate a more comprehensive gene banking effort of male adult chinook salmon gametes from listed spring and summer chinook salmon in the Snake River basin.

Goals of the cryopreservation project are: 1) preserve the genetic diversity of chinook salmon populations at high risk of extirpation through application of cryogenic techniques, 2) establish gene bank locations at independent sites for the short term, and 3) establish long term germ plasm repositories.

DESCRIPTION OF PROJECT AREA

The Nez Perce Tribe's cryopreservation project seeks to preserve Snake River spring and summer chinook salmon male gametes. The project area is the Snake River basin, specifically streams spread throughout the geographical region. Current sampling locations include: Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River stocks) and Lookingglass Hatchery (Imnaha River stock). See the project area in Figure 1.

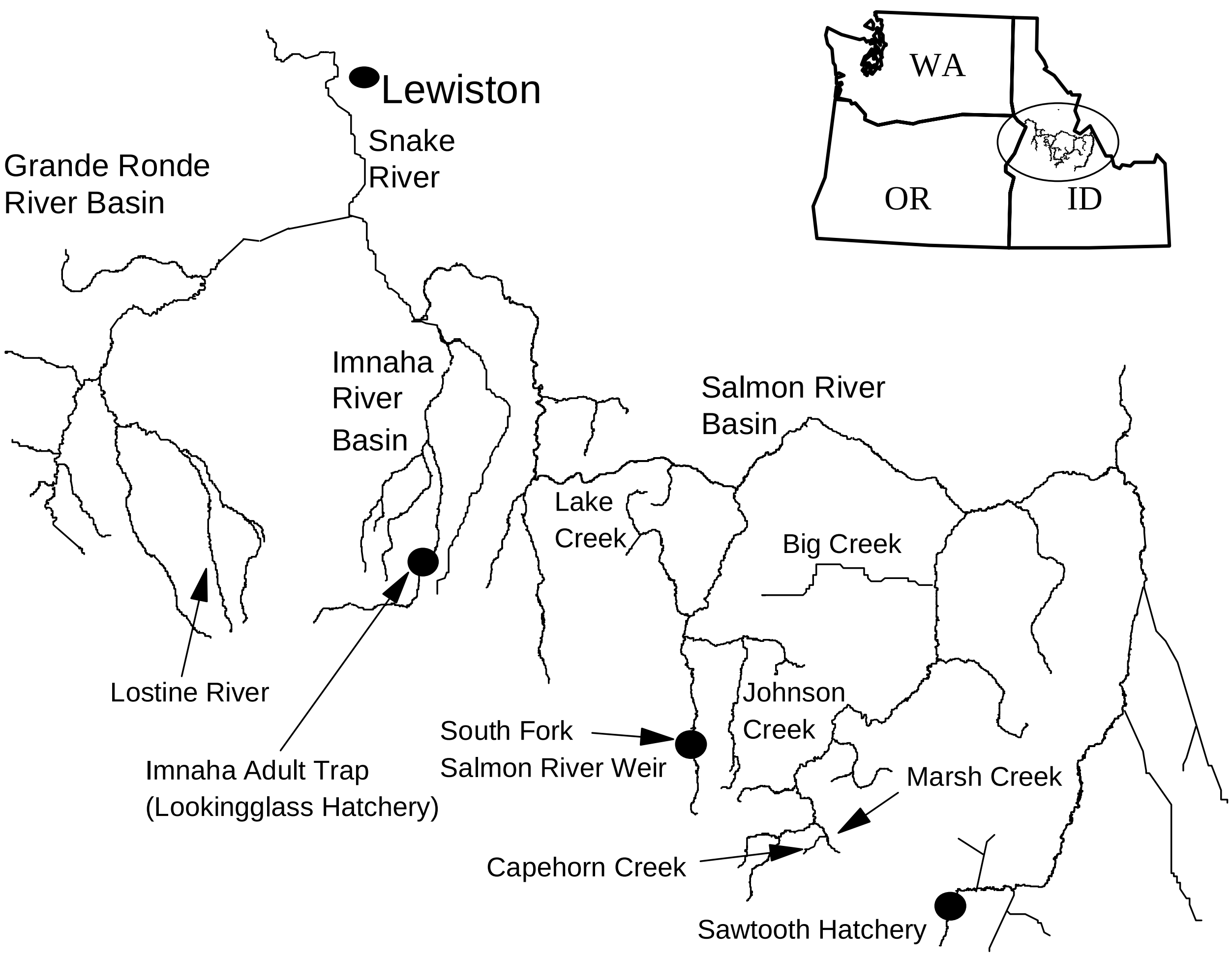

Figure 1. Map of Chinook Salmon Cryopreservation Sample Streams in the Snake River Basin in 1997.

METHODS

Fish handling protocol training was provided to all personnel prior to collection of adult male salmon to minimize stress on the fish. Each team member was assigned a specific duty to improve the efficiency of sample collection. We collected spawned-out males from the spawning grounds. All adult male salmon sampled were collected by hand or dip net.

Pre-measured MS-222 was used to anaesthetize all adult salmon, along with a sodium bicarbonate buffering compound to buffer the acidic effect of the MS-222, with the exception of unmarked fish at the South Fork Salmon River weir. Semen samples taken from natural unmarked male chinook salmon adults at the South Fork Salmon River fish weir were collected during McCall Hatchery spawning operations conducted by Idaho Department of Fish and Game. Fish handling and spawning protocols of IDFG were used and adults were not anesthetized before semen samples were taken. Extra care was taken with semen collection to ensure the quality of preserved samples. The abdomen of the anesthetized male chinook salmon was thoroughly dried to reduce or eliminate contamination of the semen samples and the milt was stripped. Some of the fish provided only enough semen for cryopreservation at one university. A few males were completely spawned out and semen samples could not be obtained. Due to a lack of storage space at the University of Idaho, samples with a motility of less than 50% were not saved.

Fish biological information (fork length and mid-eye to hypural plate length, general condition, external marks) was recorded following semen collection. Caudal fin tissue was collected for genetic (DNA) analysis. Scales were taken for age assessment and scale pattern analysis. Following sampling and data collection the anesthetized salmon were immediately returned to a slow water area and held until recovered. Semen samples were placed in two separately labeled Whirl Pak® bags, oxygenated, and placed in an insulated cooler, on newspaper over wet ice.

Samples were flown to the universities on the same day for preservation to ensure the highest quality samples possible. One Whirl Pak® bagged sample was shipped to and stored at each university as a safeguard to protect against catastrophic events that could destroy all germ plasm samples if they were stored at one facility. Cryopreservation and storage occurred independently at the University of Idaho and Washington State University within a 24 hour period. Both universities started using nitrogen vapor freezing techniques in 1997, as compared to freezing on dry ice previously.

Sperm evaluation is an important component of the cryopreservation program in order to cull poor quality sperm samples prior to freezing, and to estimate the fertility of the stored sperm post-thaw. Fertility was evaluated by sperm motility, which is the percentage of motile sperm following the addition of a sperm activating solution (Mounib 1978).

There are four stages in the cooling sequence of cryopreservation of cells (Cloud and Osborne 1997):

1) Cooling cells to the point of ice formation - this does not appear to be a critical factor in the cryopreservation of salmonid sperm;
2) The formation of ice - the goal at this stage is to have ice form near the freezing point of the extracellular solution;
3) Cooling through the critical period - there is a net movement of water out of the cells as the temperature is constantly being reduced. The cooling rate during this phase needs to be slow enough to allow water to move out of the cells, but it must be fast enough to protect the intercellular environment from the effect of the high salt concentrations. The success of cryopreservation is dependent upon required cryoprotectants (such as dimethyl sulfoxide - DMSO) in the freezing solution. These small compounds enter the cells and protect the cells during dehydration by inhibiting ice formation. The rate at which the sperm is cooled is a critical factor in the success of the cryopreservation process; For salmonid sperm, cooling rates of -20 to -30° C/minute appear to be optimal (Stoss 1983), down to approximately -79° C.
4) Reduction to liquid nitrogen temperature - the frozen milt is then plunged into liquid nitrogen at -196° C.

The amount of sperm cryopreserved varied greatly by individual fish. Many of the fish sampled had been actively spawning for several days and sometimes very little or no sperm was available. A sample of 5 ml of semen was sufficient to fill 20 0.5 ml straws, due to the dilution of semen with a freezing solution. Depending on the motility of the thawed sperm, one straw can fertilize up to 450 eggs.

Table 1. Cryopreserved samples taken from listed Snake River basin spring and summer chinook in 1997; dates collected, marked and unmarked fish numbers, fork length and % sperm motility.

Spawning Aggregate	Collection Dates	Unmarked Fish	Marked fish	Fork Length (mm)	% Sperm Motility
Lostine River	8/29	3	0	740-890	50-80
Imnaha River	8/15,22,29 9/5,10	4	42	540-920	50-100
South Fk Salmon River	8/12,19,26&9/2	13	38	690-980	50-100
Lake Creek	8/21,22	5	0	680-800	60-80
Johnson Creek	8/29	17	0	710-880	50-90
Big Creek	8/20	6	0	675-845	50-90
Capehorn Creek	8/14	5	0	635-790	50-90
Marsh Creek	8/14	4	0	710-840	70-90
Upper Salmon River	8/21,28 & 9/4	15	37	520-1055	50-100

RESULTS

Gametes from male chinook salmon were sampled from Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek, and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth and Lookingglass Hatcheries (Table 1). When sufficient semen existed, samples were collected in two separate Whirl Pak® bags, and one bag of semen delivered to the University of Idaho and the other to Washington State University for cryopreservation. These samples were frozen in 20 0.5 ml straws if the quantity allowed. Any excess semen were cryopreserved in larger 5.0 ml straws (Table 2).

Table 2. Total number of cryopreserved samples taken from listed Snake River basin spring and summer chinook in 1997; number of 0.5 ml and 5.0 ml straws in storage.

Spawning Aggregate	Total # 0.5 ml straws	Total # 5.0 ml straws	Total # straws
Lostine River	100	16	116
Imnaha River	1397	46	1443
South Fk Salmon River	1588	76	1664
Lake Creek	194	3	197
Johnson Creek	450	16	466
Big Creek	280	20	300
Capehorn Creek	130	17	147
Marsh Creek	120	18	138
Upper Salmon River	1965	175	2140
Totals	6224	387	6611

Lostine River

The Lostine River flows into the Wallowa River which empties into the Grande Ronde River. The semen samples collected from three wild fish appeared to be of good quality (no coloration or exraneous material in the sample), though the sperm motility never exceeded 80% (Table 1). This was the fourth consecutive year of cryopreservation sampling in the Lostine River system. A total of 11 cryopreserved semen samples taken from 1994 to 1997 are now in storage at the universities.

Lookingglass Fish Hatchery (Imnaha River stock)

Semen was cryopreserved from 46 Imnaha River chinook salmon that were held for spawning at the Oregon Department of Fish and Wildlife Lookingglass Fish Hatchery. This was the second year of cryopreservation sampling at the Lookingglass Fish Hatchery. A total of 79 cryopreserved semen samples taken in 1996 and 1997 are now in storage.

South Fork of the Salmon River weir

Fifty one fish were sampled over a period of four days at the South Fork Salmon River weir with Idaho Fish and Game hatchery personnel. This was the second year of cryopreservation sampling at the South Fork Salmon River weir. A total of 70 cryopreserved semen samples taken in 1996 and 1997 are now in storage.

Lake Creek

Two days were spent sampling 5 wild fish in Lake Creek. This was the second year of cryopreservation sampling in Lake Creek, a tributary of the Secesh River in the South Fork Salmon River watershed. A total of 8 cryopreserved semen samples taken in 1996 and 1997 are now in storage.

Johnson Creek

Seventeen wild salmon were sampled in one day at Johnson Creek. The Nez Perce Tribe will initiate a supplementation program in 1998 on this stream. Johnson Creek is a tributary of the East Fork South Fork Salmon River. This was the first year of cryopreservation sampling in Johnson Creek.

Big Creek

Big Creek, a tributary to the Middle Fork of the Salmon River, has been sampled for six years. Big Creek experienced three consecutive years (1994-1996) of cohort collapse and samples were not obtained. Six salmon were sampled in 1997. A total of 23 cryopreserved semen samples taken in 1992, 1993 and 1997 are now in storage.

Capehorn Creek

This was the first year of cryopreservation sampling in Capehorn Creek, a headwater stream of the

Middle Fork of the Salmon River, and five fish were sampled here in 1997.

<u>Marsh Creek</u>

This was the first year of cryopreservation sampling in Marsh Creek, a headwater stream of the Middle Fork of the Salmon River. In 1997, four fish were sampled.

<u>Sawtooth Fish Hatchery</u> <u>(upper Salmon River and East Fork Salmon River stock)</u>

Fish held in the Sawtooth Fish Hatchery from the upper Salmon River were sampled for the first time in 1997. Fish collected here were the smallest and largest length fish sampled in 1997; 52 fish were sampled, ranging from 520 to 1055 mm fork length (Table 1).

Table 3. Cryopreserved samples collected from listed Snake River basin spring and summer chinook salmon from 1992 to 1997.

Spawning Aggregate	Cryopreservation Samples, by Year						Total Samples
	1997	1996	1995	1994	1993	1992	
Lostine River	3	3	1	4	-	-	11
Imnaha River	46	33	-	-	-	-	79
S Fk Salmon R	51	19	-	-	-	-	70
Lake Creek	5	3	-	-	-	-	8
Johnson Creek	17	-	-	-	-	-	17
Big Creek	6	0	0	0	10	7	23
Capehorn Creek	5	-	-	-	-	-	5
Marsh Creek	4	-	-	-	-	-	4
Upper Salmon R	52	-	-	-	-	-	52

DISCUSSION

Semen samples from wild/natural and hatchery spring and summer chinook salmon were collected for cryopreservation at nine locations in 1997. This was the largest return year for spring and summer chinook salmon since 1978 (Fish Passage Center 1998). More semen samples for cryopreservation were collected this year than from the combined efforts of 1992 through 1996.

The three hatchery sampling locations provided 149 of the 189 samples for the cryopreservation program. Of the six stream sites, only Johnson Creek produced more than a minimal sample number. Other stream returns were not as strong and a large sample size was not obtained, this could be due to limited time at each stream and a limited effort. Big Creek spawning aggregate experienced cohort collapse in 1994, 1995 and 1996. Big Creek was surveyed in those years, but samples could not be obtained. The fish stocks sampled represent only a small portion of the stocks in the Snake River basin. The Nez Perce tribe has attempted to sample and preserve chinook salmon genetic diversity within the major subbasins in the Snake River basin.

Under normal circumstances, semen that does not have a motility rating of 50% or greater is culled. Since there is a need to store the genes from spawning aggregates that are low in abundance and at a high risk of extirpation, samples from these streams with motility ratings as low as 10% have been retained. Storage space at the University of Idaho was limited in 1997 and samples with motility ratings lower than 50% were not saved. A larger liquid nitrogen storage tank has been furnished to the University of Idaho so, in the future, more samples will be preserved.

Sampling of male chinook salmon was restricted to the later part of the spawning period to avoid harassing non spawned-out fish. By limiting the sampling period, the genetic diversity contained in early spawning fish may not be saved in the germ plasm repository. Although it would be very time consuming and labor intensive to observe and sample early spawning fish while avoiding harassment of fish that were not yet ready for spawning, it should be investigated.

The need to create a germ plasm repository becomes apparent when reviewing the number of samples being saved. Even though 1997 was a relatively good year for returning chinook salmon, the number of samples collected was minimal. In previous years, fish were not found in some streams for sampling.

RECOMMENDATIONS

The DNA from the caudal fin punches taken from most of the fish that the Nez Perce Tribe has cryopreserved the semen from may define and separate subpopulations within the Snake River basin. This analysis would indicate if the different spawning aggregates sampled are genetically different enough to be considered subpopulations. This is important because the goal is to preserve genetic diversity among the chinook salmon subpopulations in the Snake River basin. The relative frequency of polymorphism (number of loci where variation occurs) would help determine the amount of genetic diversity in the population.

It is anticipated that captive brood stock programs will be using cryopreserved semen in low adult returns years forecasted for 1998-2000. Before any cryopreserved semen is used to fertilize eggs, a genetic matrix needs to be completed from both the cryopreserved semen and captive broodstock fish to determine lineage so no directly related individuals mate and no inbreeding occurs. Population inbreeding causes a deficit of heterozygotes relative to expected Hardy-Weinberg proportions (Allendorf and Ferguson 1990). A dissimilarity matrix uses DNA analysis to look for the relative frequency of similar loci, meaning directly related individuals. Use of this analysis would provide information about breeding the most dissimilar individuals to enhance genetic diversity and prevent inbreeding depression in the subpopulations. This analysis is highly recommended before any cryopreserved semen is thawed and used in brood stock production.

Fertilization experiments are needed on the cryopreserved semen to compare fresh and frozen/thawed semen fertilization of eggs. Preliminary results of a Washington Department of Fish and Wildlife study indicates that frozen semen has a lower fertilization rate (Mendel 1996). Further study of the fertilization capacity of the cryopreserved samples is warranted.

The strategy for where the collection of semen occurs should represent a wide ecological and geographic spectrum. Therefore, the Lemhi River and Rapid River Hatchery will be added to the cryopreservation sampling sites in 1998. The Lemhi River has a spring chinook salmon run of natural and Sawtooth Hatchery fish, and 122 adult returns are forecasted for 1998 from the Lower Granite Dam counts (US v. OR Technical Advisory Committee 1998). The Lemhi River flows into the main Salmon River south of the town of Salmon in western Idaho. Rapid River Hatchery rears fish originating from the Snake River below Hells Canyon Dam. The hatchery is located on Rapid River, a tributary to the Little Salmon River. Lower Granite Dam counts estimate 2,330 adults return to the hatchery this year (US v. OR Technical Advisory Committee 1998).

The Oregon Department of Fish and Wildlife has requested the Nez Perce tribe cryopreserve steelhead semen from the fish returning in 1998 to Little Sheep Creek, a tributary of the Imnaha River, for a supplementation program. It is expected with the listing of steelhead, the proposed listing of bull trout, and the petition to list westslope cutthroat trout that more requests for cryopreserving male gametes from these species will occur. It is wise to move proactively to cryopreserve genetic diversity while the spawning aggregates of these species are relatively healthy instead of reacting to threatened levels of populations.

A liquid nitrogen vapor tank should be installed at Washington State University in 1998 to accommodate more cryopreserved samples. The tank was not purchased in 1997 and is crucial to the success of the program.

Criteria for accessing and using of cryopreserved semen samples are being developed by the Nez Perce Tribe. A central database is also being established for inventory purposes.

SUMMARY AND CONCLUSION

The cryopreservation project has a total of 269 chinook salmon semen samples in frozen storage at the University of Idaho and Washington State University. The year of 1997 was a relatively good return year and a total of 189 viable chinook salmon semen cryopreservation samples were taken from the Lostine River, Big Creek, Johnson Creek, Lake Creek, Marsh Creek and Capehorn Creek, the South Fork Salmon River weir, and Sawtooth Hatchery (upper Salmon River stock) and Lookingglass Hatchery (Imnaha River stock). A germ plasm repository for long-term and short-term storage is in place and will continue to be added to.

Collection of semen from within a stream spawning aggregate should continue until sufficient genetic diversity from that subpopulation is represented in the germ plasm repository. It is estimated that 200 individual samples are needed to establish a breeding program (Cloud personal communication). Fish in some streams such as Big Creek are low in abundance and may require a longer sampling period. Sampling of each spawning aggregate should continue until sufficient genetic material has been acquired. The goal of the gene bank is to have at least 100 samples per year from each location, covering at least five collection years.

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We thank Joe Cloud and his staff at the University of Idaho, Gary Thorgaard, Paul Wheeler and the staff at Washington State University for assistance, the long-term storage facilities and recommendations to make this a better program. We also thank the hard work and cooperation of our field crews: Glenda Claire, John Gebhards, Vonda Kirk, Eric Veach, Joe McCormack, Aaron Penney, LouAnn Laswell, Ryan Jain, David Kane, Warren Rueben, Don Bryson, and Gwen Alley. We greatly appreciate the cooperation and assistance of Gene McPherson at the Idaho Fish and Game Department McCall Fish Hatchery, Bob Lund at the Oregon Department of Fish and Wildlife Lookingglass Hatchery, and the Brent Snider at the Sawtooth National Fish Hatchery.

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APPENDIX

Appendix. Adult male chinook salmon semen collected from Lostine River, Lake Creek, Johnson Creek, Big Creek, Capehorn Creek, Marsh Creek, South Fork of the Salmon River weir, Sawtooth Hatchery (upper Salmon River stock), and Lookingglass Hatchery (Imnaha River stock); sample identification number, sperm motility, and number of 0.5 ml and 5.0 ml straws cryopreserved at the University of Idaho and Washington State University in 1997

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Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
Lostine River	29 August	NPT97-097LS	740	595
Lostine River	29 August	NPT97-098LS	810	640
Lostine River	29 August	NPT97-099LS	890	690
Lake Creek	21 August	NPT97-067LK (A)	795	635
Lake Creek	21 August	NPT97-068LK (B)	760	595
Lake Creek	21 August	NPT97-069LK (E)	680	550
Lake Creek	22 August	NPT97-080LK (F)	800	645
Lake Creek	22 August	NPT97-081LK (G)	795	635
Johnson Creek	29 August	NPT97-141JN	820	640
Johnson Creek	29 August	NPT97-142JN	750	600
Johnson Creek	29 August	NPT97-143JN	765	610
Johnson Creek	29 August	NPT97-144JN	740	585
Johnson Creek	29 August	NPT97-145JN	790	620
Johnson Creek	29 August	NPT97-146JN	735	590
Johnson Creek	29 August	NPT97-148JN	790	620
Johnson Creek	29 August	NPT97-149JN	870	680
Johnson Creek	29 August	NPT97-150JN	760	605
Johnson Creek	29 August	NPT97-151JN	800	635
Johnson Creek	29 August	NPT97-152JN	810	645

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
Johnson Creek	29 August	NPT97-153JN	880	705
Johnson Creek	29 August	NPT97-154JN	740	580
Johnson Creek	29 August	NPT97-155JN	840	670
Johnson Creek	29 August	NPT97-156JN	760	595
Johnson Creek	29 August	NPT97-157JN	710	575
Johnson Creek	29 August	NPT97-158JN	750	575
Big Creek	20 August	NPT97-044BG	790	620
Big Creek	20 August	NPT97-045BG	675	540
Big Creek	20 August	NPT97-046BG	785	610
Big Creek	20 August	NPT97-047BG	845	655
Big Creek	20 August	NPT97-048BG	780	625
Big Creek	20 August	NPT97-049BG	740	600
Capehorn Creek	14 August	NPT97-014CP	760	625
Capehorn Creek	14 August	NPT97-015CP	750	615
Capehorn Creek	14 August	NPT97-017CP	635	515
Capehorn Creek	14 August	NPT97-018CP	675	540
Capehorn Creek	14 August	NPT97-019CP	790	645
Marsh Creek	14 August	NPT97-020MR	760	605
Marsh Creek	14 August	NPT97-022MR	840	680
Marsh Creek	14 August	NPT97-023MR	780	630
Marsh Creek	14 August	NPT97-024MR	710	580
S.Fk.Salmon River	12 August	NPT97-001SF	870	720
S.Fk.Salmon River	12 August	NPT97-003SF	820	690
S.Fk.Salmon River	12 August	NPT97-008SF	770	630
S.Fk.Salmon River	12 August	NPT97-009SF	870	730
S.Fk.Salmon River	12 August	NPT97-010SF	880	740
S.Fk.Salmon River	12 August	NPT97-011SF	870	720
S.Fk.Salmon River	12 August	NPT97-012SF	780	660
S.Fk.Salmon River	12 August	NPT97-013SF	810	680

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
S.Fk.Salmon River	19 August	NPT97-026SF	830	700
S.Fk.Salmon River	19 August	NPT97-028SF	880	710
S.Fk.Salmon River	19 August	NPT97-030SF	830	670
S.Fk.Salmon River	19 August	NPT97-031SF	690	550
S.Fk.Salmon River	19 August	NPT97-032SF	980	780
S.Fk.Salmon River	19 August	NPT97-033SF	840	660
S.Fk.Salmon River	19 August	NPT97-034SF	840	680
S.Fk.Salmon River	19 August	NPT97-035SF	790	620
S.Fk.Salmon River	19 August	NPT97-036SF	790	600
S.Fk.Salmon River	19 August	NPT97-037SF	780	610
S.Fk.Salmon River	19 August	NPT97-038SF	770	610
S.Fk.Salmon River	19 August	NPT97-039SF	840	650
S.Fk.Salmon River	19 August	NPT97-040SF	770	600
S.Fk.Salmon River	19 August	NPT97-041SF	760	610
S.Fk.Salmon River	19 August	NPT97-042SF	810	630
S.Fk.Salmon River	19 August	NPT97-043SF	850	670
S.Fk.Salmon River	26 August	NPT97-082SF	860	N/A
S.Fk.Salmon River	26 August	NPT97-083SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-084SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-085SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-086SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-087SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-088SF	760	N/A
S.Fk.Salmon River	26 August	NPT97-089SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-090SF	940	N/A
S.Fk.Salmon River	26 August	NPT97-091SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-092SF	N/A	N/A
S.Fk.Salmon River	26 August	NPT97-093SF	900	N/A
S.Fk.Salmon River	26 August	NPT97-094SF	840	N/A

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
S.Fk.Salmon River	26 August	NPT97-095SF	740	N/A
S.Fk.Salmon River	26 August	NPT97-096SF	N/A	N/A
S.Fk.Salmon River	2 September	NPT97-160SF	800	N/A
S.Fk.Salmon River	2 September	NPT97-161SF	770	N/A
S.Fk.Salmon River	2 September	NPT97-162SF	740	N/A
S.Fk.Salmon River	2 September	NPT97-163SF	750	N/A
S.Fk.Salmon River	2 September	NPT97-164SF	750	N/A
S.Fk.Salmon River	2 September	NPT97-165SF	800	N/A
S.Fk.Salmon River	2 September	NPT97-166SF	N/A	N/A
S.Fk.Salmon River	2 September	NPT97-167SF	850	N/A
S.Fk.Salmon River	2 September	NPT97-168SF	820	N/A
S.Fk.Salmon River	2 September	NPT97-169SF	870	N/A
S.Fk.Salmon River	2 September	NPT97-170SF	830	N/A
S.Fk.Salmon River	2 September	NPT97-171SF	780	N/A
S.Fk.Salmon River	2 September	NPT97-172SF	810	N/A
Sawtooth Hatchery	21 August	NPT97-050ST	740	600
Sawtooth Hatchery	21 August	NPT97-051ST	740	670
Sawtooth Hatchery	21 August	NPT97-052ST	760	630
Sawtooth Hatchery	21 August	NPT97-053ST	830	660
Sawtooth Hatchery	21 August	NPT97-054ST	750	610
Sawtooth Hatchery	21 August	NPT97-055ST	780	710
Sawtooth Hatchery	21 August	NPT97-056ST	800	660
Sawtooth Hatchery	21 August	NPT97-057ST	830	660
Sawtooth Hatchery	21 August	NPT97-058ST	880	710
Sawtooth Hatchery	21 August	NPT97-059ST	870	690
Sawtooth Hatchery	21 August	NPT97-060ST	830	670
Sawtooth Hatchery	21 August	NPT97-061ST	830	660
Sawtooth Hatchery	21 August	NPT97-062ST	750	N/A
Sawtooth Hatchery	21 August	NPT97-063ST	730	N/A

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
Sawtooth Hatchery	21 August	NPT97-064ST	830	N/A
Sawtooth Hatchery	21 August	NPT97-065ST	830	N/A
Sawtooth Hatchery	21 August	NPT97-066ST	770	N/A
Sawtooth Hatchery	28 August	NPT97-100ST	780	635
Sawtooth Hatchery	28 August	NPT97-102ST	780	625
Sawtooth Hatchery	28 August	NPT97-103ST	750	600
Sawtooth Hatchery	28 August	NPT97-104ST	720	590
Sawtooth Hatchery	28 August	NPT97-105ST	820	645
Sawtooth Hatchery	28 August	NPT97-106ST	750	620
Sawtooth Hatchery	28 August	NPT97-107ST	870	665
Sawtooth Hatchery	28 August	NPT97-108ST	750	N/A
Sawtooth Hatchery	28 August	NPT97-109ST	800	N/A
Sawtooth Hatchery	28 August	NPT97-110ST	710	580
Sawtooth Hatchery	4 September	NPT97-173ST	750	610
Sawtooth Hatchery	4 September	NPT97-174ST	1050	830
Sawtooth Hatchery	4 September	NPT97-175ST	775	630
Sawtooth Hatchery	4 September	NPT97-176ST	830	665
Sawtooth Hatchery	4 September	NPT97-177ST	1070	875
Sawtooth Hatchery	4 September	NPT97-178ST	710	580
Sawtooth Hatchery	4 September	NPT97-179ST	855	690
Sawtooth Hatchery	4 September	NPT97-180ST	885	715
Sawtooth Hatchery	4 September	NPT97-181ST	740	600
Sawtooth Hatchery	4 September	NPT97-182ST	760	590
Sawtooth Hatchery	4 September	NPT97-183ST	680	560
Sawtooth Hatchery	4 September	NPT97-184ST	1055	850
Sawtooth Hatchery	4 September	NPT97-185ST	740	605
Sawtooth Hatchery	4 September	NPT97-186ST	1030	810
Sawtooth Hatchery	4 September	NPT97-187ST	725	575
Sawtooth Hatchery	4 September	NPT97-188ST	520	420

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
Sawtooth Hatchery	4 September	NPT97-189ST	990	810
Sawtooth Hatchery	4 September	NPT97-190ST	765	61
Sawtooth Hatchery	4 September	NPT97-192ST	710	550
Sawtooth Hatchery	4 September	NPT97-193ST	790	635
Sawtooth Hatchery	4 September	NPT97-194ST	850	685
Sawtooth Hatchery	4 September	NPT97-195ST	740	590
Sawtooth Hatchery	4 September	NPT97-196ST	1040	815
Sawtooth Hatchery	4 September	NPT97-197ST	830	675
Sawtooth Hatchery	4 September	NPT97-198ST	830	660
Imnaha (Lookingglass)	15 August	NPT97-025IM	900	660
Imnaha (Lookingglass)	22 August	NPT97-070IM	770	645
Imnaha (Lookingglass)	22 August	NPT97-071IM	760	630
Imnaha (Lookingglass)	22 August	NPT97-072IM	820	720
Imnaha (Lookingglass)	22 August	NPT97-073IM	750	620
Imnaha (Lookingglass)	22 August	NPT97-074IM	810	670
Imnaha (Lookingglass)	22 August	NPT97-075IM	765	625
Imnaha (Lookingglass)	22 August	NPT97-076IM	790	650
Imnaha (Lookingglass)	22 August	NPT97-077IM	750	680
Imnaha (Lookingglass)	22 August	NPT97-078IM	850	700
Imnaha (Lookingglass)	22 August	NPT97-079IM	840	700
Imnaha (Lookingglass)	29 August	NPT97-111IM	N/A	N/A
Imnaha (Lookingglass)	29 August	NPT97-114IM	800	620
Imnaha (Lookingglass)	29 August	NPT97-115IM	690	500
Imnaha (Lookingglass)	29 August	NPT97-116IM	850	690
Imnaha (Lookingglass)	29 August	NPT97-117IM	830	685
Imnaha (Lookingglass)	29 August	NPT97-118IM	830	675
Imnaha (Lookingglass)	29 August	NPT97-119IM	750	610
Imnaha (Lookingglass)	29 August	NPT97-120IM	840	660
Imnaha (Lookingglass)	29 August	NPT97-121IM	770	605

Stream	Date (1997)	Sample ID #	Fork Length (mm)	Mid-Eye Hypural (mm)
Imnaha (Lookingglass)	29 August	NPT97-122IM	920	750
Imnaha (Lookingglass)	29 August	NPT97-123IM	890	720
Imnaha (Lookingglass)	29 August	NPT97-124IM	800	640
Imnaha (Lookingglass)	29 August	NPT97-125IM	770	620
Imnaha (Lookingglass)	29 August	NPT97-126IM	850	705
Imnaha (Lookingglass)	29 August	NPT97-127IM	750	595
Imnaha (Lookingglass)	29 August	NPT97-128IM	760	620
Imnaha (Lookingglass)	29 August	NPT97-129IM	865	690
Imnaha (Lookingglass)	29 August	NPT97-130IM	820	665
Imnaha (Lookingglass)	29 August	NPT97-131IM	870	690
Imnaha (Lookingglass)	29 August	NPT97-132IM	800	630
Imnaha (Lookingglass)	29 August	NPT97-133IM	820	640
Imnaha (Lookingglass)	29 August	NPT97-135IM	855	660
Imnaha (Lookingglass)	29 August	NPT97-136IM	800	650
Imnaha (Lookingglass)	29 August	NPT97-137IM	825	660
Imnaha (Lookingglass)	29 August	NPT97-138IM	710	660
Imnaha (Lookingglass)	29 August	NPT97-140IM	770	590
Imnaha (Lookingglass)	5 September	NPT97-199IM	830	N/A
Imnaha (Lookingglass)	5 September	NPT97-200IM	830	660
Imnaha (Lookingglass)	5 September	NPT97-201IM	540	430
Imnaha (Lookingglass)	5 September	NPT97-202IM	805	655
Imnaha (Lookingglass)	5 September	NPT97-203IM	N/A	N/A
Imnaha (Lookingglass)	5 September	NPT97-204IM	760	N/A
Imnaha (Lookingglass)	5 September	NPT97-205IM	N/A	N/A
Imnaha (Lookingglass)	5 September	NPT97-206IM	910	660
Imnaha (Lookingglass)	10September	NPT97-207IM	565	470
Imnaha (Lookingglass)	10September	NPT97-208IM	910	660

Appendix 2. Sample identification number, sperm motility, and number of 0.5 ml and 5.0ml straws cryopreserved from chinook salmon at the University of Idaho and Washington State University.

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-001SF				70	20	
NPT97-003SF	90	15				
NPT97-008SF	90	15	1			
NPT97-009SF	90	20		70	20	1
NPT97-010SF	80	20		70	20	1
NPT97-011SF	90	20	2	90	20	6
NPT97-012SF	80	14		80	10	
NPT97-013SF	90	17		80	20	1
NPT97-014CP	60	20	2	10	0	
NPT97-015CP	80	20	2	10	0	
NPT97-017CP	90	20	2	0	0	
NPT97-018CP	80	20	2	50	20	2
NPT97-019CP	80	10	2	70	20	5
NPT97-020MR	80	20	2	80	20	5
NPT97-022MR	90	20	2	70	20	5
NPT97-023MR				80	20	2
NPT97-024MR	0	0		70	20	2
NPT97-025IM	50	20		100	10	
NPT97-026SF	90	20		70	20	1
NPT97-028SF	60	14		20	0	
NPT97-029SF	10	0		60	10	

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-030SF	80	20	2	80	20	3
NPT97-031SF	80	20	2	100	20	3
NPT97-032SF	80	20	2	100	20	2
NPT97-033SF	80	19		100	20	1
NPT97-034SF	70	20		70	20	
NPT97-035SF	80	19		100	20	1
NPT97-036SF	50	20		80	20	
NPT97-037SF	10	0		60	5	
NPT97-038SF	90	20		90	20	2
NPT97-039SF	80	20	2	80	20	1
NPT97-040SF	60	20		70	20	2
NPT97-041SF	70	20		1	0	
NPT97-042SF	80	7	2	60	5	
NPT97-043SF	80	20		100	20	1
NPT97-044BG	80	20	2	50	30	7
NPT97-045BG	10	0		50	30	1
NPT97-046BG	90	20	2	50	30	1
NPT97-047BG	80	20		50	30	
NPT97-048BG	80	20		70	30	1
NPT97-049BG		20	2	50	30	4
NPT97-050ST	80	11		30	0	
NPT97-051ST	90	20		60	20	2
NPT97-052ST	90	20		80	20	1

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-053ST	80	20	1	90	20	4
NPT97-054ST	60	20		80	20	4
NPT97-055ST	80	20		70	20	1
NPT97-056ST	80	20		80	20	5
NPT97-057ST	90	20	2	90	20	5
NPT97-058ST	90	20	2	70	20	3
NPT97-059ST	80	20	2	80	20	5
NPT97-069ST	90	20		80	20	
NPT97-061ST	80	20		90	20	2
NPT97-062ST	80	20		80	20	2
NPT97-063ST	90	20		90	20	5
NPT97-064ST	70	20		90	20	1
NPT97-065ST	90	20		90	20	5
NPT97-066ST	90	11		80	20	1
NPT97-067LK	70	20		80	10	
NPT97-068LK	60	12		60	2	
NPT97-069LK	80	20		70	30	2
NPT97-070IM	80	20		80	10	
NPT97-071IM	80	20		50	20	3
NPT97-072IM	90	6				
NPT97-073IM	90	13		60	20	1
NPT97-074IM	80	11		50	10	
NPT97-075IM	80	20		70	10	

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-076IM				50	9	
NPT97-077IM	90	20		60	20	1
NPT97-078IM	60	20		50	20	
NPT97-079IM	90	7				
NPT97-080LK	80	40				
NPT97-081LK				70	60	1
NPT97-082SF	80	20		80	20	3
NPT97-083SF NPT97-	80	12		50	6	
NPT97-084SF	90	20		30	0	
NPT97-085SF	80	20	2	80	20	5
NPT97-086SF NPT97-	90	11		70	10	
NPT97-087SF	80	20		50	20	3
NPT97-088SF	90	5		50	5	
NPT97-089SF NPT97-	90	20		80	20	
NPT97-090SF	70	20		80	20	3
NPT97-091SF	90	14		60	5	
NPT97-092SF	90	20	2	80	20	2
NPT97-093SF	70	20		70	20	1
NPT97-094SF	80	20	2	70	20	5
NPT97-095SF	90	20	2	60	20	3
NPT97-096SF	80	0		50	20	
NPT97-097LS	80	20	2	80	20	5
NPT97-098LS NPT97-	80	20	2	50	20	5

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-099LS	80	20	2	10	0	
NPT97-100ST	0	0		90	20	5
NPT97-102ST	80	20	2	90	20	5
NPT97-103ST	90	20	2	80	20	
NPT97-104ST	90	20		90	20	1
NPT97-105ST	80	20	2	80	20	5
NPT97-106ST	70	20		90	20	3
NPT97-107ST	80	20		80	20	3
NPT97-108ST	80	20		80	20	3
NPT97-109ST	80	20		70	20	2
NPT97-110ST	80	20	2	90	20	5
NPT97-111IM	90	20		80	20	1
NPT97-114IM	90	20		80	20	
NPT97-115IM	70	20		70	20	1
NPT97-116IM	80	20		50	20	4
NPT97-117IM	80	20		70	20	3
NPT97-118IM	80	20		70	20	2
NPT97-119IM				50	10	
NPT97-120IM	90	20		80-	14	
NPT97-121IM	90	20		90	20	2
NPT97-122IM	80	20		70	20	3
NPT97-123IM				70	5	
NPT97-124IM				80	3	

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-125IM	90	20		50	20	3
NPT97-126IM	90	20		50	20	3
NPT97-127IM	90	20		50	20	2
NPT97-128IM	80	20		70	20	1
NPT97-129IM	90	20		70	20	1
NPT97-130IM	90	20		80	20	3
NPT97-131IM	80	20		70	20	3
NPT97-132IM	90	20		20	0	
NPT97-133IM	80	20		80	20	3
NPT97-135IM	80	13		50	10	
NPT97-136IM	90	20		80	10	
NPT97-137IM	90	20		50	20	3
NPT97-138IM	90	20		50	20	3
NPT97-140IM	90	6				
NPT97-141JC	50	20		50	20	3
NPT97-142JC	90	7		5	0	
NPT97-143JC	90	20		50	20	3
NPT97-144JC	80	14		10	0	
NPT97-145JC	20	20		10	0	
NPT97-146JC	60	20		5	0	
NPT97-148JC	70	20		70	10	
NPT97-149JC	90	20		70	20	
NPT97-150JC	80	20		0	0	

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-151JC	80	20		0	0	
NPT97-152JC	70	20		70	20	1
NPT97-153JC	70	20		5	0	
NPT97-154JC	90	20		50	20	4
NPT97-155JC	80	20		70	20	5
NPT97-156JC	90	20		20	0	
NPT97-157JC	90	19				
NPT97-158JC	90	20				
NPT97-160SF	90	20		70	17	
NPT97-161SF	20	0		70	20	
NPT97-162SF				80	19	
NPT97-163SF	90	20		90	20	2
NPT97-164SF	70	13				
NPT97-165SF	70	20		90	20	
NPT97-166SF	70	19				
NPT97-167SF	80	20		90	20	1
NPT97-168SF	70	20		70	10	
NPT97-169SF	90	20		90	10	
NPT97-170SF	80	19		80	10	
NPT97-171SF	70	13		90	20	1
NPT97-172ST	80	20		80	20	5
NPT97-173ST	70	20		90	20	2
NPT97-174ST	80	20		70	20	3

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-175ST	90	20		80	20	4
NPT97-176ST	90	20		80	20	3
NPT97-177ST	30	0		50	20	3
NPT97-178ST	90	20		90	20	3
NPT97-179ST	70	20		50	20	3
NPT97-180ST				80	20	1
NPT97-181ST	80	20		50	20	3
NPT97-182ST	80	20		80	20	1
NPT97-183ST	80	20		90	20	4
NPT97-184ST	80	20		90	20	4
NPT97-185ST	90	20		80	20	3
NPT97-186ST	70	20		90	20	4
NPT97-187ST	80	20	2	90	20	3
NPT97-188ST	90	20		90	17	
NPT97-189ST	90	13		50	20	2
NPT97-190ST				100	16	
NPT97-192ST	90	20		50	19	3
NPT97-193ST	90	20	2	80	20	4
NPT97-194ST	80	20		100	20	4
NPT97-195ST	90	20		100	18	
NPT97-196ST	90	20	2	70	20	4
NPT97-197ST	20	0		80	20	4
NPT97-198ST	80	20	2	90	20	4

Sample Identification Number	Washington State University			University of Idaho		
	Sperm Motility %	#0.5ml Straws	#5ml Straws	Sperm Motility %	#0.5ml Straws	#5ml Straws
NPT97-199IM	90	20		90	20	2
NPT97-200IM				90	20	2
NPT97-201IM				90	20	2
NPT97-202IM				90	20	3
NPT97-203IM	80	20				
NPT97-205IM	90	20				
NPT97-206IM	90	20		100	20	2
NPT97-207IM	70	20	2	90	20	4
NPT97-208IM	90	20	2	80	20	4